Food Chemistry 248 (2018) 14–20

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

The fate and enantioselective behavior of zoxamide during wine-making process

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ARTICLE INFO

Keywords:
Zoxamide
Grape wine
Enantioselectivity
Processing

ABSTRACT

The fate of zoxamide and its enantiomers were evaluated in detail during wine-making process. The enantiomers of zoxamide were separated and determined by ultra-high-performance liquid chromatography coupled with tandem mass spectrometry (UHPLC–MS/MS) after each processing procedure including washing, peeling, fermentation and clarification. Significant enantioselectivity was observed in all three treatments with the half-lives of *R*-zoxamide and *S*-zoxamide estimated to be 45.6 and 52.9 h in Group A, 45.0 and 52.1 h in Group B, 56.8 and 70.7 h in Group C, respectively. The results indicated that *R*-zoxamide degraded faster than *S*-zoxamide during the fermentation process. The processing factors (PFs) of each procedure were generally less than 1, and the PF of the overall process ranged from 0.019 to 0.051, which indicated that the whole process can reduce the zoxamide residue in red and white wine obviously. The results could help facilitate more accurate risk assessments of zoxamide during wine-making process.

1. Introduction

In recent decades, the cultivation of grape is widely distribute around the world and grape is considered to be a very significant part of the modern diets (Zeying et al., 2016). Grape is not only consumed as fresh fruits, but also as processed products including raisins, wine, juice, vinegar, grape seed extract and grape seed oil (Grimalt & Dehouck, 2016). Approximately 55% of grape production is used for wine fermentation every year, and wine has become a popular alcoholic beverage due to its good flavor and many positive effects on human health, such as the decreasing of cardiovascular disease risk, and reducing damage of oxidative damage (Covas, Gambert, Fitó, & de la Torre, 2010; Holahan et al., 2012). To obtain a good-quality grape for wine making, a large amount of chemical pesticides, especially fungicides and insecticides, are applied continuously in the whole cultivation cycle of grape (Cabras & Conte, 2001; Esteve-Turrillas, Agulló, Abad-Somovilla, Mercader, & Abad-Fuentes, 2016; Herrero-Hernández et al., 2013; Pelajić, Peček, Pavlović, & Čepo, 2016; Vaquero-Fernández et al., 2008). As a result, many pesticide residues are often detected in grapes and wine, and the quality of wine may be affected (Čuš, Česnik, Bolta, & Gregorčič, 2010; Jin, Xie, Guo, & Pang, 2012; Rial, Yagüe, Cancho, & Simal, 2002). In this case, the pesticide residues existing in the final commercial wine might affect the health of consumers. In consequence, many regulations and Maximum Residue Limits (MRLs) have been developed gradually to control the pesticide residues in wine (Lu et al., 2011). In addition, a growing body research have been performed to study the detection method and dissipation rule of pesticide during wine fermentation (Lu, Shao, Dai, Diao, & Chen, 2016; Zeying et al., 2016). Unfortunately, most of the previous studies ignored the special properties of chiral pesticides and the potential safety risks caused by the chiral enantiomers. It has been estimated that 25% of pesticides currently sold are chiral, and the figure is estimated to be over 40% in China with increasing complex compounds registered for use (Williams, 1996). Usually, the enantiomers of a chiral compound have the similar physicochemical property. However, the previous study have indicated that the enantiomers have different behaviors in bioactivity, toxicity, and metabolism and dissipation (Pan et al., 2016; Zhang et al., 2015). Thus, it is of great significance to evaluate the behavior of chiral pesticide at the enantiomer level during the wine fermentation which will be conducive to more accurate food safety assessment.

Zoxamide (3,5-dichloro-N-(3-chloro-1-ethyl-1-methyl-2-oxopropyl)-4-methylbenzamide), developed by Rohm and Hass in 2001, is a non-

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https://doi.org/10.1016/j.foodchem.2017.12.052
Received 8 September 2017; Received in revised form 11 December 2017; Accepted 13 December 2017
Available online 14 December 2017
0308-8146/

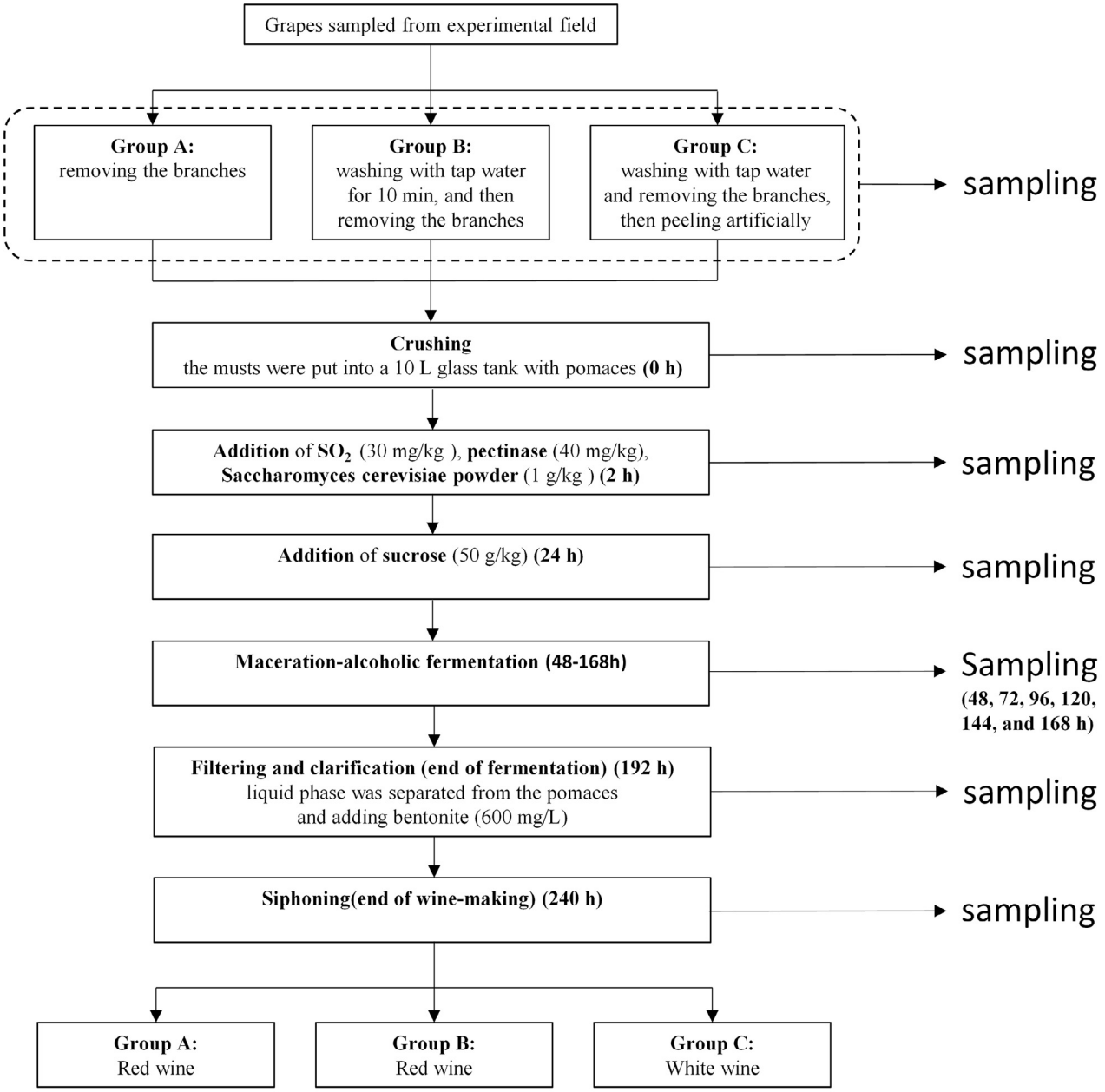

Fig. 1. Scheme for wine-making process used in this study and sampling points.

systemic typical chiral fungicide widely used in grape to control Grape Downy mildew (*Plasmopora viticola*) (Mei et al., 2014). The property of zoxamide including water solubility, octanol-water coefficient and Henry's constant are 0.681 mg/L, 5.75×10^{3} and 6.59×10^{-3}, respectively. The current MRLs of zoxamide in grape is 5 mg/kg. It acts after spore germination to inhibit tubulin polymerization and arrests nuclear division by binding to the β-subunit, resulting in the suppression of germ-tube-elongation and inhibiting fungal penetration (Bi et al., 2011; Cai et al., 2015). Zoxamide has one asymmetrically substituted C atom, which bring to two different enantiomeric configurations: *R*-zoxamide and *S*-zoxamide. JMPR report indicated that zoxamide could induce polyploidy in an assay for chromosomal aberration in Chinese hamster ovary cells in vitro (ZOXAMIDE 487-522 JMPR 2007). Besides, the processing factors (PFs), the ratio of residue levels in processed products and their respective raw products, is very important in the dietary intake assessment of related pesticides in processed commodities (Amvrazi & Albanis, 2008; Han et al., 2014). When the processing produce leads to an increase of the residue level, the PFs would be used to recommend MRLs for processed products with an existing Codex commodity code (Fao, Who, & AGP, 2006; Gonzálezrodríguez, Rialotero, Canchogrande, Gonzalezbarreiro, & Simalgándara, 2011). To our knowledge, no report has been performed to evaluate the enantioselective dissipation and PFs of zoxamide enantiomers in wine-making process.

The current study was therefore aimed at investigating the enantioselective dissipation of zoxamide enantiomers during the red wine and white wine fermentation and evaluating the PFs of each processing procedure including washing, peeling, fermentation and clarification. The result from this study may provide more accurate information for evaluating the wine safety induced by zoxamide.

2. Materials and methods

2.1. Chemicals and reagents

The analytical standard zoxamide (enantiomer ratio = 1:1,

chemical purity = 99.3%) and commercial zoxamide product (8.3% WG) named "Jia Er Wo" were both obtained from Gowan Company (Guangzhou, China). LC-grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Analytical-grade acetonitrile, sodium chloride (NaCl), and anhydrous magnesium sulfate ($MgSO_4$) were purchased from Beijing Chemical Company (Beijing, China). PSA (40 μm) and GCB (40 μm) were obtained from Agela Technologies (Beijing, China). Ultrapure water was prepared with a Milli-Q reagent water system (Millipore, Bedford, MA, USA).

The standard stock solution (100 mg/kg) of racemic zoxamide was prepared in LC-grade acetonitrile. The working solution and calibration were prepared by appropriate dilution of the stock solution at the concentration of 0.01, 0.05, 0.1, 0.5, 1.0, and 5.0 mg/L on the day of analysis. Correspondingly, the matrix-matched standard solution were prepared at the same concentration by adding blank sample extracts to each serially diluted standard solution. All the solutions were stored at 4 °C in the dark.

2.2. Field experiments

The field trials were carried out under greenhouse conditions, which were located at the experimental base of the Institute of Plant Protection, Chinese Academy of Agricultural Sciences (Langfang, China, 116.4 °E, 39.3 °N). Four 30 m^2 trial plots were selected, three replicates and one control were performed in experimental plots and where zoxamide had never been previously applied. According to the OECD guideline for the pesticide residue in processed commodities (Test No. 508: Magnitude of the Pesticide Residues in Processed Commodities, 2007), the *rac*-zoxamide commercial product (8.3% WG) was used with the foliar spraying mode at 62 g of active ingredient per hectare (3 times of recommended dose). The pesticides was applied three times with a seven day interval and 50 kg grape samples were collected at 3 days after the last treatment. All the samples were transported to the laboratory and processed immediately.

2.3. Winemaking and sampling

The winemaking experiment were carried out in three groups (Group A, B, C) as shown in Fig. 1. About 5 kg grapes were used in each treatment. Different treatments were performed before crushing procedure. Group A: removing the branches. Group B: washing with tap water for 10 min, and then removing the branches after the surface dried. Group C: washing with tap water and removing the branches, then peeling artificially. Then, the same procedures were carried out in three groups. Firstly, the grapes were crushed and the musts were put into a 10 L glass tank with pomaces (skins and seeds). Then, 30 mg/kg of SO_2 and 40 mg/kg of pectinase was added and 1 g/kg of Saccharomyces cerevisiae powder was added to the must, respectively. After 24 h, about 50 g/kg of sucrose was added to the must. The fermentation tanks were placed with the temperature kept at 25 ± 1 °C during maceration and alcoholic fermentation. In order to improve maceration effect, stirring the must three times a day in the first three days. After a week, the liquid phase was separated from the pomaces by filtering, and the alcoholic fermentation was completed. Subsequently, 600 mg/L bentonite was added for clarification. Siphoning the clear wine after two days clarification. Finally, red wine was obtained from Group A and B, white wine was obtained from Group C.

For each group, three repetitions were prepared, sampling was conducted starting with the raw grapes until to the end of clarification (240 h) as shown in Fig. 1. And the quantities of yeast in each sample were counted use microscope. All the obtained samples were stored at −20 °C until analysis.

2.4. Sample preparation

A portion of 10 g homogenized samples (grape, pomaces, and 10 mL for must or wine) were weighted into a 50 mL polypropylene centrifuge tube with a screw cap. Then 10 mL ACN was added, and the samples were shaken vigorously for 10 min. Afterwards, 1 g NaCl and 4 g anhydrous MgSO4 were added, and the shaking step was again conducted for 3 min. The tubes were centrifuged for 5 min at 2811 × *g* relative centrifugal force. Next, 1.5 mL of the upper layer was transferred into a single-use centrifuge tube containing the sorbents (20 mg GCB + 50 mg PSA + 150 mg anhydrous MgSO4), and then vortexed for 1 min and centrifuged for 5 min at 2400 × *g* relative centrifugal force. Finally, the supernatants were filtered (0.22 μm nylon syringe filter) into an autosampler vial for detection.

2.5. Instrument conditions

Chiral chromatographic separation of the zoxamide enantiomer was carried out with an Acquity UHPLC system (Waters, MA, USA), including an Acquity UHPLC binary solvent manager, an Acquity UHPLC sample manager and an Acquity UHPLC column heater equipped with an Lux Amylose-2 chiral column (150 mm × 2 mm, 3 μm particle size, Phenomenex, USA). A mobile phase of acetonitrile and water (70:30, v/v) with a flow rate of 0.5 mL/min and the total analysis time was 5 min. The temperature of the column oven and sample manager were set at 25 °C and 15 °C.

Detection was achieved using a triple-quadrupole mass spectrometer (Waters, MA, USA) equipped with an ESI source, operating in positive mode. The typical parameters including capillary voltage, source temperature, desolvation temperature, con gas flow, and desolvation gas flow were 3.0 kV, 150 °C, 350 °C, 50 L/h and 600 L/h. The optimized MRM parameters for zoxamide enantiomers detection were as follows: the cone voltage of zoxamide was set at 35 V, *m/z* 336.18 → 187.00 and *m/z* 336.18 → 159.00 were selected as the quantitative ion and qualitative ion with the corresponding collision energy 46 and 12 V, respectively. Masslynx V.4.1 was used to collect and analyze the obtained data. Basing on the instrument conditions and our previous report (Pan et al., 2017), the elution order of the zoxamide enantiomers was (−)-*R*-zoxamide (1.59 min) and (+)-*S*-zoxamide (2.00 min), respectively.

2.6. Method validation

The method was validated to evaluate the performance according to the conventional validation procedure, which including specificity, linear range, limit of quantification (LOQ), matrix effect, accuracy and precision. Blank samples (grapes, pomaces and wine) were extracted and analyzed to verify the absence of interfering species at the retention time of zoxamide enantiomers. A six-point standard solution and matrix-matched standard solutions were prepared at the concentration of 10, 50, 100, 500, 1000, 5000 μg/L and analyzed to evaluate the linearity of the method. The LOQ of each enantiomer was estimated as the minimum spiked concentration with suitable recovery (70–120%) and precision (RSD < 20%). The matrix effect is considered as a major disadvantage in the LC–MS/MS analysis because the co-eluted impurity could compete with target compound for charge carriers during the ionization process (Pan et al., 2015). Especially in chiral analysis, different matrix effect on each enantiomer could cause different peak intensity which might bring difficulties to precise quantification. In this study, matrix effect for each enantiomer in different matrix was considered and calculated as follows: matrix effect (ME%) = [(slope of calibration curves in matrix − slope of calibration curves in solvent)/slope of calibration curves in solvent] × 100%.

The recovery assay were performed to evaluate the accuracy and precision of the sample preparation method. For these, 10 g blank samples (10 mL for wine) were spiked with the standard solution at 10, 100, 1000, 5000 μg/kg of each enantiomer. The spiked samples were vortexed for 30 s and let to stand for 30 min to make sure the target compound penetrate into the matrices evenly. Then, the extraction and

purification procedures were conducted according to the aforementioned method. Each spiked concentration was processed with five replicates in three days.

2.7. Data analysis

The fermentation trial were performed in triple. Data were statistically evaluated by one-way ANOVA analysis with SAS 9.0 software.

The dissipation kinetics of zoxamide enantiomers during fermentation, beginning with crushing (0 h) and ending with clarification (192 h), were estimated using the first-order kinetics equation. The half-life of each enantiomer was measured using the following equations:

$$C = C_0 e^{-kt} \quad (1)$$

$$T_{1/2} = ln2/k \quad (2)$$

where C_0 and C indicated the concentration of the enantiomer at initial time and time t. k is the dissipation rate constant.

The enantiomeric fraction (EF) was used to investigate the enantioselective dissipation of the zoxamide enantiomers during fermentation. EF was described as the following equation:

$$EF = \frac{(+)\text{-}S\text{-}zoxamide}{(+)\text{-}S\text{-}zoxamide + (-)\text{-}R\text{-}zoxamide} \quad (3)$$

where (+)-*S*-zoxamide and (−)-*R*-zoxamide represent the concentration of the specified enantiomer.

The Joint FAO/WHO Meeting on Pesticide Residue (JMPR) evaluate food processing data on residue behavior where significant residue occur in plant or plant products which are processed into food. Processing factors (PFs) are calculated to show the effect on residue levels and the disposition on the residues in the various processed products.

$$PFs = \frac{residue \text{ level in processed } commodity}{\text{residue level in the raw agricultural commodities or commodity to be processed}} \quad (4)$$

The PFs values < 1 indicate that a reduction of residue occurs in the processed commodity, while the values > 1 indicate that the concentration effect occurs in the processing procedures.

3. Results and discussion

3.1. Method validation

Blank samples were performed to evaluate the specificity of the selected ion chromatograms and no peaks were observed in the retention time of each enantiomer. The linearity was evaluated by preparing four different calibration curves (solvent, grape, pomaces and wine) with the concentration range of 10–5000 μg/L for each enantiomer. Satisfactory linearity was observed with the correlation coefficient (R^2) range from 0.9992 to 0.9999. The LOQ was estimated to be 10 μg/kg for each enantiomer in three different matrices. The matrix effect was carefully investigated, and the different signal enhancements were observed in three kinds of matrices with the range of 10.8–57.2%. Generally, matrix effect value greater than 10% or smaller than −10% should not be ignored (Pan et al., 2016). So external matrix-matched standards were generated to quantify real samples in order to obtained more accurate results. The mean recoveries of zoxamide enantiomers in three matrices were within 89.3–115.1% at four concentration level (Table 1). The reproducibility of the recovery study was expressed as relative standard deviation (RSD) and the RSD value were ranged from 0.4% to 9.2%. The result confirmed that the method was able to obtain accurate quantitative data in this study.

Table 1
Recoveries and RSDs of zoxamide enantiomer in grape samples at four fortification levels (n = 5).

Sample	Fortification (μg/kg)	*R*-zoxamide			*S*-zoxamide		
		Recovery	RSD	LOQ	Recovery	RSD	LOQ
Grape	10	106.9	7.2	10	106.1	6.2	10
	100	101.4	2.2	(μg/	100.2	2.0	(μg/
	1000	98.0	3.3	kg)	97.1	2.5	kg)
	5000	92.4	1.3		90.3	0.9	
Wine	10	115.1	3.5	10	106.1	1.7	10
	100	110.0	3.3	(μg/	105.7	1.2	(μg/
	1000	103.1	4.1	kg)	100.8	2.7	kg)
	5000	97.6	0.8		93.5	0.4	
Pomace	10	99.4	1.2	10	99.0	0.7	10
	100	94.7	2.4	(μg/	93.2	1.3	(μg/
	1000	97.8	4.7	kg)	90.9	9.2	kg)
	5000	90.1	3.2		89.3	6.3	

3.2. Effects of processing

The corresponding concentration of zoxamide enantiomers and racemate in processed commodity are presented in Table 2. In general, washing is the first step in most processing procedures and many reports have investigated the effect of washing process to eliminate pesticide residues in agricultural commodity (Han et al., 2013; Kaushik, Satya, & Naik, 2009). In this study, the raw grape was washed with tap water for 10 min. And as shown in Table 2, the mean loss of (−)-*R*-zoxamide, (+)-*S*-zoxamide, and *rac*-zoxamide were 67.0%, 67.6%, and 67.3%, respectively after washing process. Comparing to the previous report, washing process showed stronger removal capability of zoxamide, with the 5.71% loss of pyridaben and 16.0% loss of difenoconazole (Han et al., 2014; Kong et al., 2012). This may be because zoxamide has the relatively lower octanol/water partition coefficient (Log P = 3.76) and weaker absorbability, compared with pyridaben (Log P = 6.37) and difenoconazole (Log P = 4.36). A majority of zoxamide remained in the surface of skin and easy to be washed. Peeling is another important step in the processing procedure of winemaking. The data showed that peeling has a significant effect on the reduction of (−)-*R*-zoxamide, (+)-*S*-zoxamide, and *rac*-zoxamide with 93.2%, 93.4% and 93.3% decrease, respectively. The result was similar to the previous studies with other pesticides in agricultural commodities processing, in which the eliminate rate of pesticide residue through peeling was in the range of 60–100% (Amvrazi, 2011; Cengiz, Certel, Karakas, & Gocmen, 2007). The residue level of (−)-*R*-zoxamide, (+)-*S*-zoxamide, and *rac*-zoxamide in grape skin was 45137.7, 42074.1, 87211.8 μg/kg, respectively (without washing procedure). The value was far higher than the residue in peeled grape, which indicated that zoxamide was primarily deposited on the grape skin. This may be due to cuticular wax serving as the transport barrier (Riederer & Schreiber, 1995). In addition, the result demonstrated that peeling was more effective than washing in removing zoxamide residues. This because washing step only eliminate the pesticide residues which are loosely attached to the surface, while peeling even could remove the pesticide that have penetrated into the skin of grape (Rawn et al., 2008).

The wine-making process includes fermentation process and clarification process and the fermentation begins with the pressing of the grapes. In this study, wine-making process were carried out with skins (Group A and B) and without skins (Group C). In Group A and B, the wine were made with all of the residue on the grapes, while in Group C, the process include only the residues that had passed into the sarcocarp. After pressing process, the pesticide came into a biphasic system consisted of a liquid phase (the must) and a solid phase (cake and lees), and it would be apportioned between the two phases (Cabras & Angioni, 2000). As illustrated in Table 2, the residue of (−)-*R*-zoxamide, (+)-*S*-

Table 2
The concentration of zoxamide enantiomer of grape samples after different process (n = 3).

Treatments	Compounds	Concentration (μg/kg)						
		Raw grapes	Washed grape	Peeled grape	Grape skin	Fermentation wine	Byproduct (pomace)	Clarification wine
Group A	*Rac*-zoxamide	12046.1 ± 280.3	–	–	–	638.2 ± 58.1	9414.7 ± 341.4	627.5 ± 0.01
	R-zoxamide	6242.4 ± 143.8	–	–	–	246.0 ± 26.5	2518.3 ± 169.3	240.4 ± 11.8
	S-zoxamide	5803.7 ± 136.5	–	–	–	392.2 ± 31.6	6896.4 ± 272.1	387.1 ± 23.6
Group B	*Rac*-zoxamide	12046.1 ± 280.3	3940.6 ± 96.9	–	–	173.8 ± 10.9	3107.6 ± 205.3	161.0 ± 16.5
	R-zoxamide	6242.4 ± 143.8	2061.3 ± 30.2	–	–	67.0 ± 8.1	667.3 ± 68.2	61.0 ± 8.3
	S-zoxamide	5803.7 ± 136.5	1879.3 ± 66.7	–	–	106.8 ± 2.7	2440.3 ± 137.1	100.0 ± 8.2
Group C	*Rac*-zoxamide	12046.1 ± 280.3	–	810.8 ± 36.1	87211.8 ± 508.3	50.3 ± 5.5	506.9 ± 57.3	42.2 ± 5.7
	R-zoxamide	6242.4 ± 143.8	–	427.4 ± 15.3	45137.7 ± 231.9	19.4 ± 2.5	135.1 ± 17.8	15.6 ± 2.8
	S-zoxamide	5803.7 ± 136.5	–	383.4 ± 20.8	42074.1 ± 276.4	30.9 ± 3.0	371.8 ± 39.5	26.6 ± 2.9

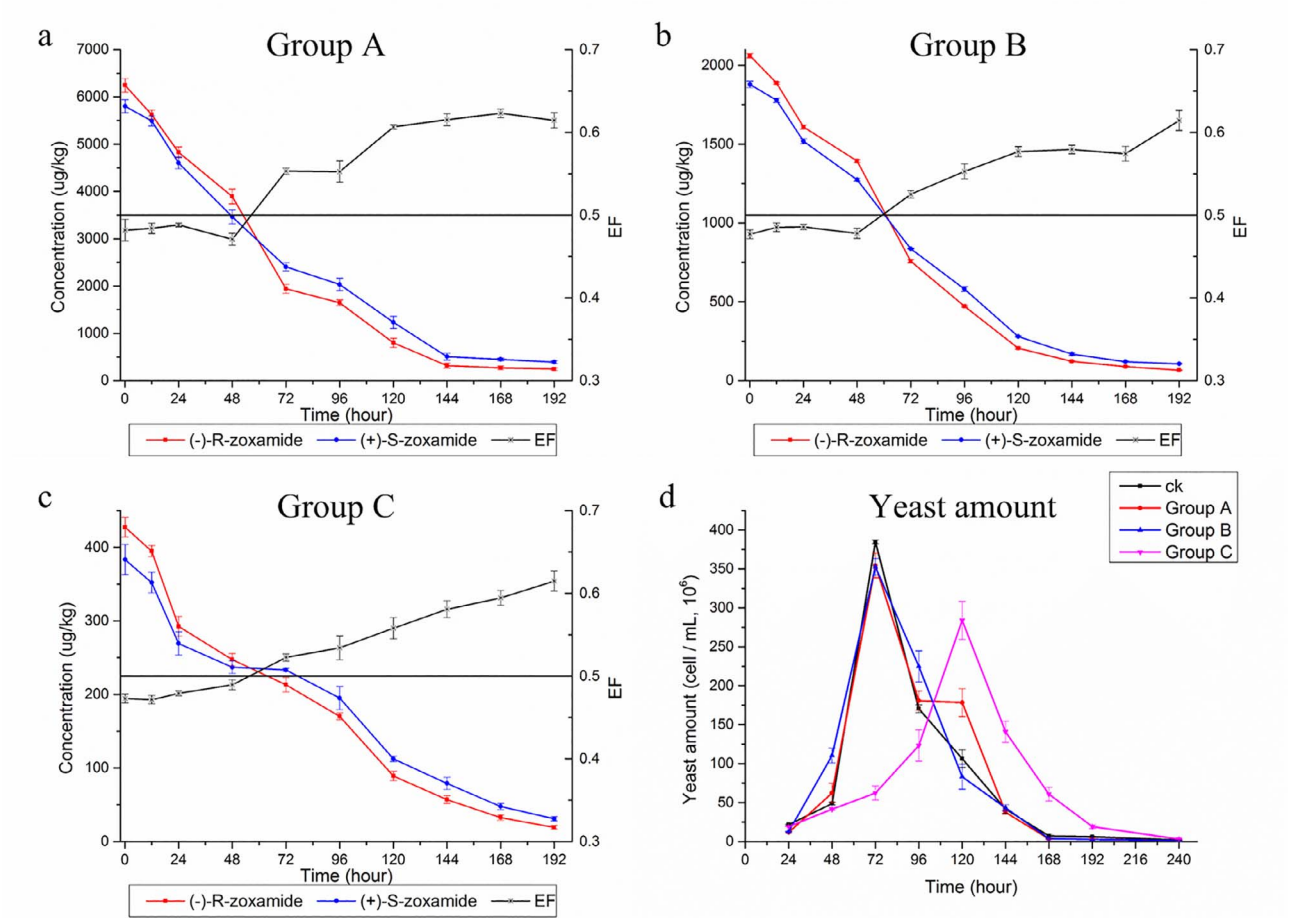

Fig. 2. Concentration and EF value of zoxamide enantiomers (a, b, c) and yeast amount (d) during wine-making process.

Table 3
First-order constants (*k*), half-lives, correlation coefficients (R^2), and Statistical P value for the dissipation of zoxamide enantiomer during fermentation process.

Treatments	Compounds	*k* (h^{-1})	Half-live (h)	R^2	P
Group A	*R*-zoxamide	0.0152	45.6	0.9778	0.0242
	S-zoxamide	0.0131	52.9	0.9861	
Group B	*R*-zoxamide	0.0154	45.0	0.9771	0.0197
	S-zoxamide	0.0132	52.1	0.9621	
Group C	*R*-zoxamide	0.0122	56.8	0.9729	0.0091
	S-zoxamide	0.0098	70.7	0.9479	

zoxamide, and *rac*-zoxamide in wine was reduced 92.0–98.9% under three kind of treatment after the fermentation process. However, the residues of zoxamide in byproducts (cake and lees) were remarkably higher than that in wine. This might be due to the reason that the high octanol/water partition coefficient generate a great affinity of zoxamide for the solid phase and zoxamide were significantly adsorbed on the cake and lees. In industrial production, wine, cake and lees were used to produce alcoholic beverages. For example, 1 L of brandy (45° alcoholic content) is prepared from 4.5 L of wine at 10% alcohol. 1 L of grappa (45° alcoholic content) is prepared from 10 kg of cake at 4.5% alcohol (Cabras & Angioni, 2000). Thus, the potential risk of zoxamide in by-product should be further investigated in industrial production. In addition, the zoxamide residue decreased slightly after adding bentonite for clarification. It was mainly because of the weaker adsorption

Table 4
PFs for different processing procedure of zoxamide (n = 3).

Treatments	Compounds	PFs of processing types				
		Washing	Peeling	Fermentation	Clarification	Overall process
Group A	*Rac*-zoxamide	–	–	0.034	0.968	0.033
	R-zoxamide	–	–	0.026	0.962	0.025
	S-zoxamide	–	–	0.044	0.972	0.043
Group B	*Rac*-zoxamide	0.324	–	0.029	0.912	0.026
	R-zoxamide	0.327	–	0.021	0.896	0.019
	S-zoxamide	0.321	–	0.037	0.922	0.034
Group C	*Rac*-zoxamide	–	0.059	0.046	0.826	0.038
	R-zoxamide	–	0.060	0.034	0.795	0.027
	S-zoxamide	–	0.057	0.060	0.846	0.051

capacity of bentonite.

3.3. Enantioselective dissipation of zoxamide in wine-making process

Generally, the enantiomers of chiral compound often show different dissipation rate when exposed to biological systems. In this study, the addition yeast and must comprise the wine-making biological systems. As shown in Fig. 2, in three kind of fermentation process, the concentration of (−)-*R*-zoxamide was higher than (+)-*S*-zoxamide at the beginning of fermentation. This is due to the preferential dissipation of (+)-*S*-zoxamide in the field trial and the result is similar to our previous study (Pan et al., 2017). Then, the enantiomers gradually decreased with time elapse. The corresponding dissipation kinetics and half-lives of zoxamide enantiomers are shown in Table 3. The dissipation of zoxamide enantiomer in fermentation process followed the first-order of kinetics (R^2 = 0.9702, 0.9704 in Group A, R^2 = 0.9714, 0.9760 in Group B and R^2 = 0.9426, 0.9611 in Group C). The half-lives of (−)-*R*-zoxamide and (+)-*S*-zoxamide were 45.6, 52.9 h in Group A, 45.0, 52.1 h in Group B, and 56.8, 70.7 h in Group C. The half-lives of zoxamide enantiomers were significantly different ($P < .05$, student's paired *t*-test). Contrary to the higher concentration of (−)-*R*-zoxamide in unprocessed grape, the concentration of (+)-*S*-zoxamide was higher than that of (−)-*R*-zoxamide in red and white wine.

The EF value was measured to evaluate the stereoselective dissipation of zoxamide enantiomers in three kind of fermentation process. As shown in Fig. 2, the EF value had the similar variation trend, EF increased gradually from 0.482, 0.477, and 0.473 to 0.617, 0.621, and 0.630 in Group A, B and C during the whole fermentation process. The results indicated that (−)-*R*-zoxamide were preferentially degraded in three kind of fermentation and resulting in relative enrichment of (+)-*S*-zoxamide in red and white wine. The previous reports also found the enantioselective dissipation phenomenon of chiral compound in fermentation process. Lu et al. found (−)-*S*-diclofop-methyl degraded faster than the *R*-isomer during alcohol fermentation process (Lu et al., 2011). Enzymatic systems have been generally regarded to play a significant role in the enantioselective process of many chiral compound. In this study, enantioselective dissipation trend was similar. However, the dissipation rate of zoxamide in Group C was significant slower than that in Group A and B, and there was no significant difference in Group A and B. The significant difference in dissipation rate may be due to the difference of the yeast amount. As shown in Fig. 2d, the total quantities of yeasts in control, Group A and Group B reached maximum value at 72 h, while the yeast amount reached highest at 120 h in Group C. This could be because the grape skin (in control, Group A and Group B) floating on the top as insulation reduced heat dissipating, and the higher temperature in fermentation tanks accelerate the reproduction of yeast. Then, the high yeast amount may accelerate the dissipation of zoxamide. The previous reports also found the similar result, which demonstrated that the yeasts had the ability to degrade some pesticides and reduce the residue content in grape wine (Cabras & Angioni, 2000). Though the half-lives (45.0–70.7 h) was short comparing the whole processing time (240 h), the concentration of zoxamide in the final grape wine were also high (Table 2) and the grape wine safety risk cannot be ignored. The result may provide a basis for the risk assessments of zoxamide in wine-making process. Nevertheless, further studies should be performed to clarify the mechanism of enantioselectivity during fermentation process.

3.4. Processing factors

The PFs of zoxamide racemate and enantiomers during wine-making process were evaluated and presented in Table 4. The results demonstrated that PFs were generally less than 1, which indicated that each process procedure had the effect of reducing zoxamide residue. The PFs of the overall process for racemate and enantiomers of zoxamide ranged from 0.019 to 0.051 in three treatments, indicating that the whole process could reduce the zoxamide in red and white wine obviously. The results were similar to the previous reports, which presented that wine-making processes (washing, pressing, fermentation, clarification) could reduce the concentration of pesticide residues to some extent (Angioni, Dedola, Garau, Schirra, & Caboni, 2011; Gonzálezrodríguez, Canchogrande, & Simalgándara, 2011). In addition, it is important to bear in mind that washing is an essential procedure during wine-making process in order to reduce the food risks.

4. Conclusion

In this study, the residue change of zoxamide racemate and enantiomers in grapes samples during wine-making processing was carefully evaluated. Different processing procedures could affect the reduction of zoxamide residue. Peeling process has a significant effect on the reduction of zoxamide, because a great amount of zoxamide were retained on grape skin. The results demonstrated that the PFs after each processing procedure were generally less than 1. The PFs of the overall process for zoxamide ranged from 0.019 to 0.051, which indicated that the whole process can reduce the zoxamide residue in red and white wine obviously. In addition, the enantioselectivity of zoxamide enantiomers were investigated during the fermentation process. Significant enantioselectivity was observed in all three treatment with the similar enantioselective dissipation trend. (−)-*R*-zoxamide was preferentially degraded and resulting in relative enrichment of (+)-*S*-zoxamide in red and white wine. The results might provide more accurate risk assessments of zoxamide in wine-making process.

Acknowledgement

This work was supported by the National Key Research and Development Program of China (2016YFD0200200).

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